Flavonoid Glycosides with Antioxidant Activity from the Petals of *Carthamus tinctorius*

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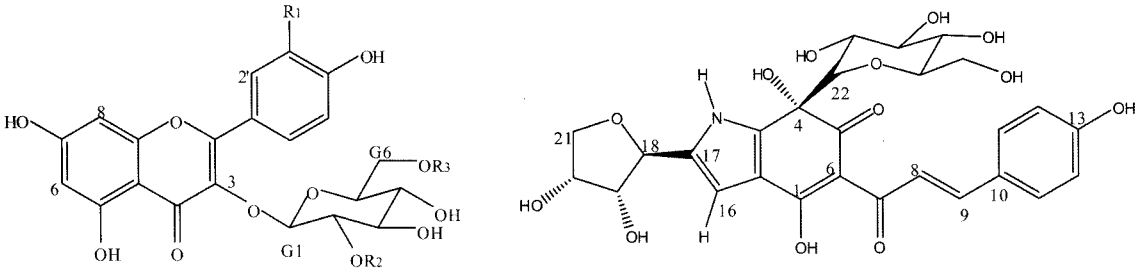
The flavonoid glycosides in the petals of safflower (*Carthamus tinctorius* L., Compositae) have been used as useful sources of natural yellow and red colorants. The colorants so far reported from *C. tinctorius* are carthamin [Obara and Onodera , 1979], safflomin A [Onodera *et al.*, 1981], safflor yellow A [Takahashi *et al.*, 1982], safflor yellow B [Takahashi *et al.*, 1984], safflomin C [Onodera *et al.*, 1989], hydroxysafflor yellow A [Meselhy *et al.*, 1993], tinctormine [Meselhy *et al.*, 1993], precarthamin [Kumazawa *et al.*, 1994], anhydrosafflor yellow B [Kazuma *et al.*, 2000], and cartormin [Yin and He, 2000]. All these constituents are classified as members of the *C*-glucosylquinochalcone family of flavonoids, a unique structure that occurs only in *C. tinctorius* [Kazuma *et al.*, 2000]. As a part of our ongoing search for the chemical constituents from *C. tinctorius*, we isolated carthamin, hydroxysafflor yellow A, safflor yellow B, and precarthamin and examined their pH and thermal stability [Kim and Paik, 1997; Yoon *et al.*, 2003]. Enzymatic conversion of the yellow-colored precarthamin to the red-colored carthamin by a purified enzyme obtained from the fresh yellow petals of *C. tinctorius* has also been reported [Cho *et al.*, 2000]. The natural yellow food colorant isolated from the safflower contains, besides C-glucosylquinochalcones, other flavonoid glycosides [Kazuma *et al*, 2000; Hattori *et al.*, 1992]. Flavonoids are naturally occurring polyphenolics with antioxidant activities proposed to contribute to the human health.

From the BuOH-soluble fraction of MeOH extract of the dried safflower petals, we isolated flavonol glycosides (**1-3**) and C-glucosylquinochalcone (**4**) (Fig. 1). The radical-scavenging activity of the compounds against ABTS radical is described in this paper.

The dried petals of *C. tinctorius* (5 kg) were extracted at



	R_1	R_2	R_3
1	H	glucosyl	H
2	OH	H	rhamnosyl
3	H	H	rhamnosyl

Fig. 1. Structures of the isolated flavonoid glycosides 1-4.

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Abbreviations: ABTS, 2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid)diammonium salt

Table 1. ^{1}H NMR Spectroscopic Data of Compounds 1-3 in CD_3OD

Proton	1	2	3
6	6.18 d (2.0)	6.20 d (2.0)	6.20 d (2.0)
8	6.38 d (2.0)	6.38 d (2.0)	6.39 d (2.0)
2'	8.03 d (8.8)	7.66 d (2.0)	8.05 d (8.8)
3'	6.90 d(8.8)		6.88 d (8.8)
5'	6.90 d (8.8)	6.86 d (8.4)	6.88 d (8.8)
6'	8.03 d (8.8)	7.62 dd (8.4, 2.0)	8.05 d (8.8)
G1	5.42 d (8.0)	5.10 d (7.6)	5.12 d (7.2)
G2	3.74 t (8.0)	3.47 m	3.42 m
G3	3.60 d (8.8)	3.41 m	3.41 m
G4	3.30 m	3.25 m	3.25 t (7.6)
G5	3.21 m	3.34 m	3.34 m
G6	3.69 d (12.4)	3.80 d (11.2)	3.80 d (10.0)
	3.48 dd (12.0, 5.6)	3.39 m	3.38 m
G1'	4.76 d (7.2)		
G2'	3.36 m		
G3'	3.43 m		
G4'	3.39 m		
G5'	3.33 m		
G6'	3.79 dd (12.0, 2.0)		
	3.31 m		
R1		4.52 brs	4.51 d (2.4)
R2		3.63 m	3.63 m
R3		3.53 m	3.52 dd (9.6, 3.6)
R4		3.27 m	3.27 t (8.0)
R5		3.45 m	3.44 m
R6		1.11 d (6.4)	1.11 d (6.4)

Table 2. ^{13}C NMR Spectroscopic Data of Compounds 1-3 in CD_3OD

Carbon	1	2	3
2	157.61	158.02	159.25
3	133.65	134.35	135.38
4	178.35	178.06	179.20
5	161.78	161.64	162.80
6	98.60	98.72	99.89
7	164.53	164.67	165.81
8	93.49	93.65	94.84
9	157.17	157.17	158.36
10	104.57	104.38	105.57
1'	121.56	121.85	122.63
2'	131.07	116.44	132.25
3'	114.98	144.53	116.03
4'	160.21	148.50	161.31
5'	114.98	114.80	116.03
6'	131.07	122.30	132.25
G1	99.78	103.49	104.52
G2	81.23	74.54	75.71
G3	77.02	76.98	78.09
G4	69.91	70.20	71.40
G5	76.69	76.01	77.50
G6	61.28	67.37	68.53
G1'	103.43		
G2'	74.33		
G3'	77.06		
G4'	70.10		
G5'	76.69		
G6'	61.43		
R1		101.18	102.33
R2		70.91	72.04
R3		71.04	72.26
R4		72.74	73.85
R5		68.53	69.69
R6		16.79	17.96

room temperature with 80% aqueous MeOH (20 L × 2). After filtration and evaporation of the solvent under reduced pressure, the combined crude extract was suspended in water and then successfully partitioned with ethyl acetate (EtOAc) and butanol (BuOH) to afford EtOAc-soluble and BuOH-soluble fractions. The BuOH-soluble fraction was subjected to Sephadex LH-20 using 20~80% CH_3CN/H_2O gradient to yield four fractions. The fourth fraction (5.2 g) was subjected to HPLC with 20~40% CH_3CN/H_2O (ODS column: 250 × 10 mm; flow rate: 1 mL/min; 40 min) to afford four subfractions. Further purification of these subfractions with 20~40% aqueous CH_3CN yielded three flavonols (**1-3**) and one quinochalcone (**4**).

Compound **1** (12.3 mg): R_t 26.027 min; R_f 0.65 (BuOH/EtOH/H_2O = 4 : 1 : 2); UV (MeOH) 347 (log ε 3.83), 267 (log ε 3.92) nm; IR (KBr) 3383, 1657, 1608 cm^{-1}; FABMS *m/z* 633.1 $[M+Na]^+$, 611.0 $[M+H]^+$; LCMS *m/z* 633.6 $[M+Na]^+$, 611.6 $[M+H]^+$, 449.4 $[M-162+H]^+$, 287.4 $[M-162-162+H]^+$; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2. Through comparison with the reported data [Kazuma *et al.*, 2000; Price *et al.*, 1998; Baek *et al.*, 1998], compound **1** was determined to be kaempferol 3-*O*-sophoroside.

Compound **2** (13.6 mg): R_t 27.787 min; R_f 0.64 (BuOH/EtOH/H_2O = 4 : 1 : 2); UV (MeOH) 355 (log ε 3.76), 257 (log ε 3.85) nm; IR (KBr) 3392, 1654, 1607 cm^{-1}; FABMS *m/z* 632.9 $[M+Na]^+$, 610.9 $[M+H]^+$, 303.1 $[M-146-162+H]^+$; LCMS *m/z* 633.6 $[M+Na]^+$, 611.5 $[M+H]^+$, 465.4 $[M-146+H]^+$, 303.4 $[M-146-162+H]^+$; ^{1}H NMR data, see Table 1; ^{13}C NMR data, see Table 2. The chemical structure of **2** was determined to be quercetin 3-*O*-rutinoside as revealed through the comparison with the reported data [Kazuma *et al.*, 2000].

Compound **3** (49.6 mg): R_t 31.667 min; R_f 0.69 (BuOH/

Table 3. NMR Spectroscopic Data of Compounds 4 in CD_3OD

	^1H	^{13}C	HMBC (C→H)
1		186.56	16
2		116.31	16
3		141.21	16
4		78.25	G2
5		196.85	
6		108.96	
7		180.97	9
8	7.50 d (16.0)	118.22	
9	7.76 d (16.0)	142.68	8, 11, 15
10		127.07	9, 12, 14
11, 15	7.53 d (8.8)	130.53	9, 8
12, 14	6.81 d (8.8)	115.61	11, 15
13		160.09	11, 15, 12, 14
16	6.51 s	103.28	18
17		134.33	16, 18, 19
18	4.69 d (7.6)	77.02	21, 19, 16
19	4.16 dd (7.6, 4.8)	76.39	18, 20, 21
20	4.26 m	71.2	21
21	4.23 m	73.09	
	3.81 dd (9.6, 2.0)		
G1	3.40 d (9.6)	84.62	G2, G3
G2	3.58 t (9.2)	69.7	G1, G3
G3	3.28 m	78.73	G2, G4
G4	3.31 m	69.41	G3, G5, G6
G5	3.01 m	79.86	G6
G6	3.67 d (3.2)	61.13	

EtOH/H_2O = 4 : 1 : 2); UV (MeOH) 349 (log ε 3.85), 266 (log ε 3.93) nm; IR (KBr) 3352, 1655, 1607 cm^{-1}; FABMS *m/z* 617.7 $[M+Na]^+$, 595.7 $[M+H]^+$, 449.1 $[M-146+H]^+$, 287.1 $[M-146-162+H]^+$; LCMS *m/z* 617.5 $[M+Na]^+$, 595.5 $[M+H]^+$, 449.4 $[M-146+H]^+$, 287.3 $[M-146-162+H]^+$; ^1H NMR data, see Table 1; ^{13}C NMR data, see Table 2. Compound **3** was established as kaempferol 3-*O*-rutinoside [Kazuma *et al.*, 2000].

Compound **4** (42.4 mg): R_t 35.107 min; R_f 0.62 (BuOH/EtOH/H_2O = 4 : 1 : 2); UV (MeOH) 406 (log ε 4.03), 245 (sh), 221 (log ε 3.78) nm; IR (KBr) 3366, 1652, 1600 cm^{-1}; FABMS *m/z* 598.4 $[M+Na]^+$; LCMS *m/z* 598.6 $[M+Na]^+$, 576.6 $[M+H]^+$, 414.5 $[M-162+H]^+$; ^1H and ^{13}C NMR and HMBC data in CD_3OD, see Table 3. The structure of Compound **4,** showing a characteristic absorption maximum at 406 nm, was consistent with that of cartormin [Yin and He, 2000].

Measurement of the radical-scavenging activities of compounds **1-4** was determined based on the decolorization of the ABTS radical at 734 nm [van den Berg *et al.*, 1999; Huang *et al.*, 2005]. The results showed the radical-scavenging activities of compounds **1-4** and Trolox, the standard reference compound, had dose-dependent effects on the ABTS radical. Trolox suppressed the absorbance of the ABTS radical with EC_{50} value of 14.4 ± 0.22 μM, while the EC_{50} values of compounds **1-4** were 4.07 ± 0.09, 5.26 ± 0.10, 6.08 ± 0.03, and 4.63 ± 0.10 μM, respectively (Table 4), suggesting these compounds have strong free radical-scavenging activities. Quercetin 3-rutinoside **2** showed slightly higher antioxidant activity than kaempferol 3-rutinoside **3**, probably due to the dihydroxyl group in its B ring.

Table 4. ABTS radical-scavenging activities of flavonoid glycosides 1-4 and Trolox

Compound	EC_{50} (μM)
1	4.07 ± 0.09
2	5.26 ± 0.10
3	6.08 ± 0.03
4	4.63 ± 0.10
Trolox	14.4 ± 0.22

In conclusion, three flavonol glycosides, kaempferol 3-sophoroside **1**, quercetin 3-rutinoside **2**, kaempferol 3-rutinoside **3**, and one C-glucosylquinochalcone, cartormin **4**, were isolated from the dried petals of safflower using reversed phase HPLC. Isolated flavonoid glycosides **1-4** showed strong antioxidant activities against the ABTS radical system.

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